| T7 | lacO | RBS | ATG | Biotag | EK | attR1 | Cm^R | ccdB | attR2 | T7 term |

pET104-DEST™
7618 bp

lacI

Ampicillin

T7 prom

pBR322 ori

Fig. 1

```
CAAGGAGATGGCGCCCAACAGTCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCTC
ATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCAC
CTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAATTA
ATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTA
AGAAGGAGATATACATATGGGCGCCGGCCACCCCGGTGACCGCCCCGCTGGCGGGCACTATCTGGAAGGTG
CTGGCCAGCGAAGGCCAGACGGTGGCCGCAGGCGAGGTGCTGCTGATTCTGGAAGCCATGAAGATGGAAA
CCGAAATCCGCGCCGCGCAGGCCGGGACCGTGCGCGGTATCGCGGTGAAAGCCGGCGACGCGGTGGCGGT
CGGCGACACCCTGATGACCCTGGCGGGCTCTGGATCCGATCTGTACGACGATGACGATAAGGGAATTATC
ACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTT
TGCATAAAAAACAGACTACATAATACTGTAAAAACAACAATATCCAGTCACTATGGCGGCCGCATTAGGC
ACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGAGTTAGGATCCGGCGAGAT
TTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATG
GCATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAGCTG
GATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTC
TTGCCCGCCTGATGAATGCTCATCCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGA
TAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATAC
CACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCT
ATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTT
TGATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAA
GGCGACAAGGTGCTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTCTGTGATGGCTTCCATGTCGGCA
GAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAACGCGTGGATCCGGCTT
ACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCACCGGTGCTAGCGTATACCCGAAGTATGTCAAAA
AGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACACGCACAGCTATCAGTTGCTCAAGGCATA
TATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGCAGAATGAAGCCCGTCGTCTGCGTGCCGAAC
GCTGGAAAGCGGAAAATCAGGAAGGGATGGCTGAGGTCGCCCGGTTTATTGAAATGAACGGCTCTTTTGC
TGACGAGAACAGGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGAGACCGTTATCGTCT
GTTTGTGGATGTACAGAGTGATATTATTGACACGCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGCA
CGTCTGCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCA
TGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCG
CGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAATGTCAGGCTCCGTTATACAC
AGCCAGTCTGCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTA
TGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTTTCTTGTACAAAG
TGGTGATAATTAATTAAGATAGCTCAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGC
TGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTG
AAAGGAGGAACTATATCCGGATATCCCGCAAGAGGCCCGGCAGTACCGGCATAACCAAGCCTATGCCTAC
AGCATCCAGGGTGACGGTGCCGAGGATGACGATGAGCGCATTGTTAGATTTCATACACGGTGCCTGACTG
CGTTAGCAATTTAACTGTGATAAACTACCGCATTAAAGCTAGCTTATCGATGATAAGCTGTCAAACATGA
GAATTAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAAT
GGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAA
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGA
AGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTT
TGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATC
GAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCA
CTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCG
CATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATG
ACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAA
CGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCG
TTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCA
ACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGA
TGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAA
```

Fig. 2A

```
ATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT
ATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAG
GTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAA
ACTTCATTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAA
CGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTT
TTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCA
AGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTA
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCC
TGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACC
GGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTAC
ACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACA
GGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA
TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGG
CGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTC
ACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATAC
CGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGG
TATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATATATGGTGCACTCTCAGTACAATCTGCTC
TGATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGAC
ACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGT
GACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCTGCGG
TAAAGCTCATCAGCGTGGTCGTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGA
GTTTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCCTGTTT
GGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGGTAATGATACCGATGAAACGAGAGAGG
ATGCTCACGATACGGGTTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAAACAACTGG
CGGTATGGATGCGGCGGGACCAGAGAAAAATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGT
AGGTGTTCCACAGGGTAGCCAGCAGCATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGAC
TTCCGCGTTTCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACG
TTTTGCAGCAGCAGTCGCTTCACGTTCGCTCGCGTATCGGTGATTCATTCTGCTAACCAGTAAGGCAACC
CCGCCAGCCTAGCCGGGTCCTCAACGACAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCAACGCTG
CCCGAGATGCGCCGCGTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGGGTTGGTTT
GCGCATTCACAGTTCTCCGCAAGAATTGATTGGCTCCAATTCTTGGAGTGGTGAATCCGTTAGCGAGGTG
CCGCCGGCTTCCATTCAGGTCGAGGTGGCCCGGCTCCATGCACCGCGACGCAACGCGGGGAGGCAGACAA
GGTATAGGGCGGCGCCTACAATCCATGCCAACCCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGT
GACGATCAGCGGTCCAGTGATCGAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGA
TGGTCGTCATCTACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAA
GAATCATAATGGGGAAGGCCATCCAGCCTCGCGTCGGCGAACGCCAAGACGTAGCCCAGCGCGTCGGC
CGCCATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGA
GCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGT
CCTCGCCGAAAATGACCCAGAGCGCTGCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCAT
AAGTGCGGCGACGATAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGC
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TCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCG
TATTGGGCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTG
GCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTG
GTTAACGGCGGGATATAACATGAGCTGTCTTCGGTATCGTCGTATCCCACTACCGAGATATCCGCACCAA
CGCGCAGCCCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGC
AGTGGGAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCT
TCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCG
CCGAGACAGAACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCCAC
GCCCAGTCGCGTACCGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGA
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Fig. 2B

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AATAACGCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAA
TGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCTTCGACGCCGCTTCG
TTCTACCATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGC
GACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTT
GTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGA
AACGTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGACATCG
TATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGC
GAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGA
AGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCCAAGGAATGGTGCATG
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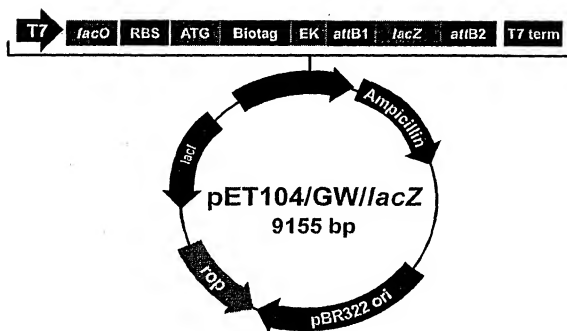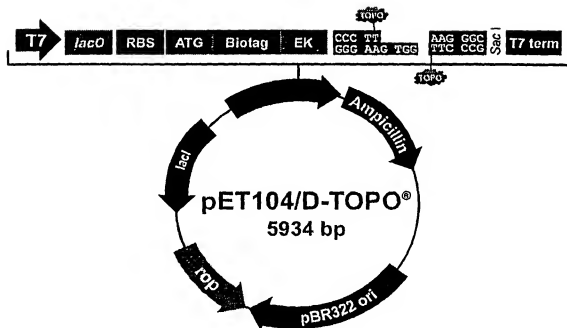
Fig. 2C

| T7 | *lacO* | RBS | ATG | Biotag | EK | *attB1* | *lacZ* | *attB2* | T7 term |

pET104/GW/*lacZ*
9155 bp

lacI

Ampicillin

rop

pBR322 ori

Fig.3

Fig. 4

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CAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCTC
ATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCAC
CTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAATTA
ATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTA
AGAAGGAGATATACATATGGGCGCCGGCACCCCGGTGACCGGCCCCGGCCGGCGGCGGCACTATCTGGAAGGTG
CTGGCCAGCGAAGGCCAGACGGTGGCCGCAGGCGAGGTGCTGCTGATTCTGGAAGCCATGAAGATGGAAA
CCGAAATCCGCGCCGCGCAGGCCGGGACCGTGCGCGGTATCGCGGTGAAAGCCGGCGACGCGGTGGCGGT
CGGCGACACCCTGATGACCCTGGCGGGCTCTGGATCCGATCTGTACGACGATGACGATAAGGGAATTGAT
CCCTTCACCAAGGGCGAGCTCAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCC
ACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTGCTGAAAG
GAGGAACTATATCCGGATATCCCGCAAGAGGCCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCA
TCCAGGGTGACGGTGCCGAGGATGACGATGAGCGCATTGTTAGATTTCATACACGGTGCCTGACTGCGTT
AGCAATTTAACTGTGATAAACTACCGCATTAAAGCTAGCTTATCGATGATAAGCTGTCAAACATGAGAAT
TAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTT
TCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATAC
ATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAG
TATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCT
CACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAAC
TGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTT
TAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATA
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TAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGAT
CGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGG
GAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAA
CGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGA
GGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCT
GGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGACTGCGGGGCCAGATGGTAAGCCCTCCCGTATCG
TAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGC
CTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTT
CATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTG
AGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCT
GCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAG
CTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGT
AGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTT
ACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGAT
AAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCG
AACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTA
TCCGGTAAGCGGCAGGGTCGGGACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTT
TATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGA
GCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACAT
GTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCT
CGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATCGGTTAT
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GCCGCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCC
GCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACC
GTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCTGCGGTAAA
GCTCATCAGCGTGGTCGTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGAGTTT
CTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCCTGTTTGGTC
ACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGGTAATGATACCGATGAAACGAGAGAGGATGC
TCACGATACGGGTTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAAACAACTGGCGGT
```

Fig. 5A

```
ATGGATGCGGCGGGACCAGAGAAAAATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGT
GTTCCACAGGGTAGCCAGCAGCATCCTGCGATGCAGATCCGGGAACATAATGGTGCAGGGCGCTGACTTCC
GCGTTTCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTT
GCAGCAGCAGTCGCTTCACGTTCGCTCGCGTATCGGTGATTCATTCTGCTAACCAGTAAGGCAACCCCGC
CAGCCTAGCCGGGTCCTCAACGACAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCCCG
AGATGCGCCGCGTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGGGTTGGTTTGCGC
ATTCACAGTTCTCCGCAAGAATTGATTGGCTCCAATTCTTGGAGTGGTGAATCCGTTAGCGAGGTGCCGC
CGGCTTCCATTCAGGTCGAGGTGGCCCGGCTCCATGCACCGCGACGCAACGCGGGGAGGCAGACAAGGTA
TAGGGCGGCGGCCTACAATCCATGCCAACCCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACG
ATCAGCGGTCCAGTGATCGAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGT
CGTCATCTACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAAGAAT
CATAATGGGGAAGGCCATCCAGCCTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGCGCGTCGGCCGCC
ATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGAGCGA
GGGCGTGCAAGATTCCGAATACCGCAAGCGACAGCCGCGATCATCGTCGCGCTCCAGCGAAAGCGGTCCTC
GCCGAAAATGACCCAGAGCGCTGCCGGCCACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGT
GCGGCGACGATAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCG
GTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCA
GTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATT
GGGCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCC
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CAGCCCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTG
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AGTCGCGTACCGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATA
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CAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCTTCGACGGCCGCTTCGTTCT
ACCATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACG
GCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGC
CACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACG
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ACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAA
GGTTTTGCGGCCATTCGATGGTGTCCGGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCA
GCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGTGCATG
```


Fig. 5B

| T7 | lacO | RBS | ATG | Biotag | EK | lacZ | T7 term |

pET104/D/*lacZ*
9006 bp

lacI

Ampicillin

rop

pBR322 ori

Fig.6

Fig.7

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GACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTT
AAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACA
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ATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCG
CCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCC
AAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTA
TGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGC
AGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAA
TGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACG
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CTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGTTTAAACTT
AAGCTTACCATGGGCGCCGGCACCCCGGTGACCGCCCCGCTGGCGGGCACTATCTGGAAGGTGCTGGCCA
GCGAAGGCCAGACGGTGGCCGCCAGGCGAGGTGCTGCTGATTCTGGAAGCCATGAAGATGGAAACCGAAAT
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AAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAGCTGGATATTA
CGGCCTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCG
CCTGATGAATGCTCATCCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTT
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AGGTGCTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTCTGTGATGGCTTCCATGTCGGCAGAATGCT
TAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAACGCGTGGATCCGGCTTACTAAAA
GCCAGATAACAGTATGCGTATTTGCGCGCTCGCGAACCGGTGTATACCCGAAGTATGTCAAAAAGAGGTG
TGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCAGCTATCAGTTGCTCAAGGCATATATGATG
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AACAGGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGAGCCGTTATCGTCTGTTTGTG
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TGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGAC
CACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGAGTGGCTGATCTCAGCCACCGCGAAAAT
GACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAATGTCAGGCTCCGTTATACACAGCCAGT
CTGCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAA
TCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTTTCTTGTACAAAAGTGGTGAT
AATTAATTAAGATCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGC
CATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTA
ATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGGTGGGGCAG
GACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTG
AGGCGGAAAGAACCAGCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGC
GGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTC
TTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGCATCCCTTTAGGGT
TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCC
ATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTC
```

Fig. 8A

CAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGGGGATTTCGG
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TTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCA
GCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGT
CAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCC
GCCCCATGGCTGACTAATTTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAG
AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTT
TCGGATCTGATCAGCACGTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAG
GTGAGGAACTAAACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAACGGCTA
CAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAGCGACGGCCGCAT
CTTCACTGGTGTCAATGTATATCATTTTACTGGGGGACCTTGTGCAGAACTCGTGGTGCTGGGCACTGCT
GCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGCGATCGGAAATGAGAACAGGGGCATCTTGAGCC
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CGTGGCCGAGGAGCAGGACTGACACGTGCTACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTG
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CTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTT
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CTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGC
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TTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCC
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CAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGC
CGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGA
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CTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTG
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GGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCA
CATTTCCCCGAAAAGTGCCACCTGACGTC

Fig. 9

T7 **ATG** **Biotag** **EK site** | Asp718 I / Kpn I | CCC TT / GGG AAG TGG | TOPO | AAG GGC / TTC CCG | Xba I / Apa I | TOPO

P_CMV — BGH pA — SV40 ori — EM7 — Blasticidin — SV40 pA — pUC ori — Ampicillin
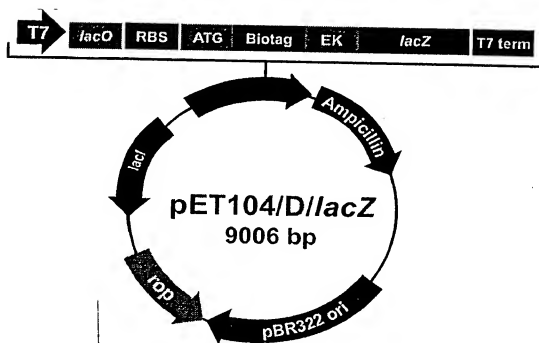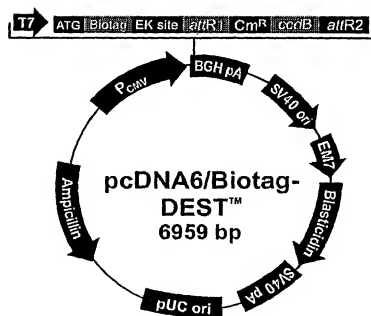
pcDNA6/Biotag™/
D-TOPO®
5302 bp

Fig. 10

```
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GCGAAGGCCAGACGGTGGCCGCAGGCGAGGTGCTGCTGATTCTGGAAGCCATGAAGATGGAAACCGAAAT
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```


Fig. II A

```
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CTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGAT
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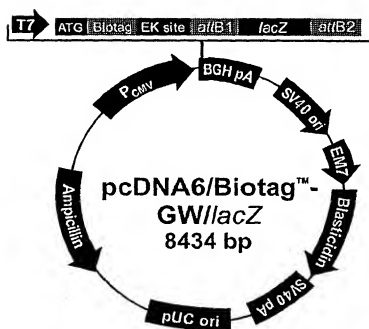

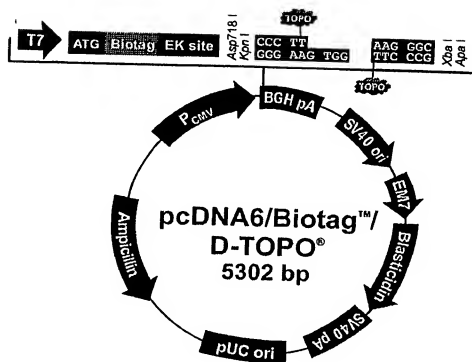
Fig. II B

Fig. 12

| ATG | Biotag | EK site | *att*R1 | Cm^R | *ccdB* | *att*R2 |

P_MT · SV40 pA · Ampicillin · pUC ori

pMT/Biotag-
DEST™
5375 bp

Fig. 13

```
TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAGTGAATTAATTCGTTGCAGGA
CAGGATGTGGTGCCCGATGTGACTAGCTCTTTGCTGCAGGCCGTCCTATCCTCTGGTTCCGATAAGAGAC
CCAGAACTCCGGCCCCCCACCGCCCACCGCCACCCCCATACATATGTGGTACGCAAGTAAGAGTGCCTGC
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AGAGGTGAATCGAACGAAAGACCCGTGTGTAAAGACCGCGTTTCCAAAATGTATAAAACCGAGAGCATCTG
GCCAATGTGCATCAGTTGTGGTCAGCAGCAAAATCAAGTGAATCATCTCAGTGCAACTAAAGGGGGGATC
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GGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATG
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Fig. 14A

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TGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCC
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CATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT
```
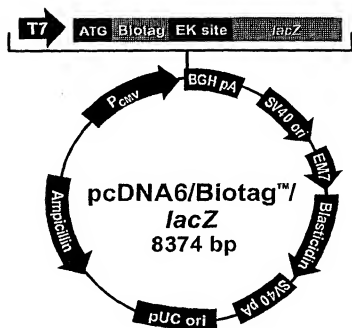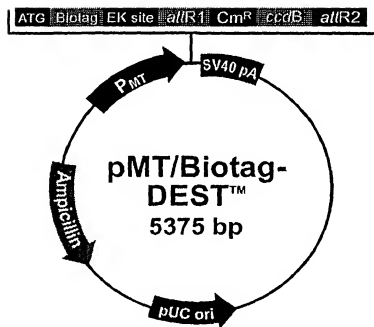

Fig. 14B

ATG | Biotag | EK site | attB1 | lacZ | attB2

P_MT · SV40 pA

pMT/Biotag/
GW-*lacZ*
6993 bp

Ampicillin

pUC ori
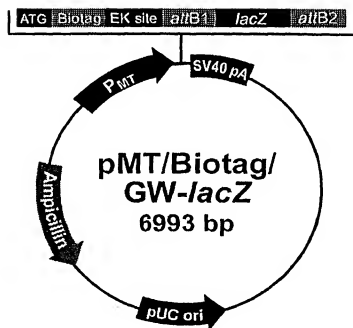
Fig. 15

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pET104-DEST™ vector by recombination. Non-shaded regions are derived from the pET104-DEST™ vector.
- Bases 568 and 2230 of the pET104-DEST™ sequence are marked.
- The biotin binding site is labeled with a *.

```
121   ATAGGCGCCA GCAACCGCAC CTGTGGCGCC GGTGATGCCG GCCACGATGC GTCCGGCGTA GAGGATCGAG ATCTCGATCC
                          T7 promoter/priming site
             T7 promoter                          lac operator
201   CGCGAAATTA ATACGACTCA CTATAGGGGA ATTGTGAGCG GATAACAATT CCCCTCTAGA AATAATTTTG TTTAACTTTA
                                                                        Biotag™
         RBS                      Met Gly Ala Gly Thr Pro Val Thr Ala Pro Leu Ala Gly Thr Ile Trp Lys Val
281   AGNAGGAGAT ATACAT ATG GGC GCC GGC ACC CCG GTG ACC GCC CCG CTG GCG GGC ACT ATC TGG AAG GTG

      Leu Ala Ser Glu Gly Gln Thr Val Ala Ala Gly Glu Val Leu Leu Ile Leu Glu Ala Met Lys Met
351   CTG GCC AGC GAA GGC CAG ACG GTG GCC GCA GGC GAG GTG CTG CTG ATT CTG GAA GCC ATG AAG ATG
                                                                                          Biotin binding site

      Glu Thr Glu Ile Arg Ala Ala Gln Ala Gly Thr Val Arg Gly Ile Ala Val Lys Ala Gly Asp Ala
417   GAA ACC GAA ATC CGC GCG GCG CAG GCC GGG ACC GTG CGC GGT ATC GCG GTG AAA GCC GGC GAC GCG
                                      Biotag™ forward priming site
                                                                                      EK recognition site
      Val Ala Val Gly Asp Thr Leu Met Thr Leu Ala Gly Ser Gly Ser Asn Leu Tyr Asp Asp Asp Asp
483   GTG GCG GTC GGC GAC ACC CTG ATG ACC CTG GCG GGC TCT GGA TCC GAT CTG TAC GAC GAT GAC GAT
                                          568                                        2230
      Lys Gly Ile Ile Thr Ser Leu Tyr Lys Lys Ala Gly ... ... ***
549   AAG GGA ATT ATC ACA AGT TTG ...                             ... TTGTACAAAG
        CCT TAA TAG TGT TCA AAC ATG TTT ...           GENE           ...TTTC
      EK cleavage site                attB1                                    attB2
                                                                              T7 reverse priming site
2241  TGGTGATAAT TAATTAAGAT AGCTCAGATC CGGCTGCTAA CAAAGCCCGA AAGGAAGCTG AGTTGGCTGC TGCCACCGCT
      ACCACTATTA
2321  GAGCAATAAC TAGCATAACC
```

Fig. 16

Fig. 17

Flow Chart

The flow chart below describes the general steps required to clone and express your blunt-end PCR product.

```
        ┌─────────────────────────────────────┐
        │     Determine strategy for PCR        │
        └─────────────────────────────────────┘
                          │
                          ▼
        ┌─────────────────────────────────────┐
        │    Produce blunt-end PCR product      │
        │  using properly designed PCR primers  │
        └─────────────────────────────────────┘
                          │
                          ▼
    ┌───────────────────────────────────────────────┐
    │            TOPO® Cloning Reaction:              │
    │  Mix together PCR product and pET104/D-TOPO® vector │
    └───────────────────────────────────────────────┘
                          │   Incubate 5 minutes
                          ▼   at room temperature
        ┌─────────────────────────────────────┐
        │    Transform into TOP10 E. coli cells │
        └─────────────────────────────────────┘
                          │
                          ▼
        ┌─────────────────────────────────────┐
        │      Select and analyze colonies       │
        └─────────────────────────────────────┘
                          │
                          ▼
        ┌─────────────────────────────────────┐
        │   Choose a positive transformant and   │
        │         isolate plasmid DNA            │
        └─────────────────────────────────────┘
                          │
                          ▼
        ┌─────────────────────────────────────┐
        │      Transform BL21 Star™(DE3)         │
        │   and induce expression with IPTG      │
        └─────────────────────────────────────┘
```
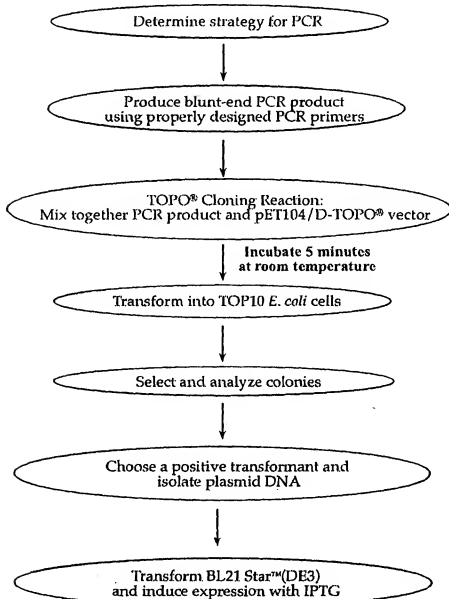
Fig. 18

```
121  ATAGGCGCCA GCAACCGCAC CTGTGGCGCC GGTGATGCCG GCCACGATGC GTCCGGCGTA GAGGATCGAG ATCTCGATCC
                         T7 promoter/priming site
     |    T7 promoter    |            |        lac operator                  |
201  CGCGAAATTA ATACGACTCA CTATAGGGGA ATTGTGAGCG GATAACAATT CCCCTCTAGA AATAATTTTG TTTAACTTTA
       RBS                                                          Biotag™
     |         |                                            |
281  AGAAGGAGAT ATACAT ATG GGC GCC GGC ACC CCG GTG ACC GCC CCG CTG GCG GGC ACT ATC TGG AAG GTG
                      Met Gly Ala Gly Thr Pro Val Thr Ala Pro Leu Ala Gly Thr Ile Trp Lys Val

351  CTG GCC AGC GAA GGC CAG ACG GTG GCC GCA GGC GAG GTG CTG CTG ATT CTG GAA GCC ATG AAG ATG
     Leu Ala Ser Glu Gly Gln Thr Val Ala Ala Gly Glu Val Leu Leu Ile Leu Glu Ala Met Lys Met
                                                                                    Biotin binding site
417  GAA ACC GAA ATC CGC GCC GCG CAG GCC GGG ACC GTG CGC GGT ATC GCG GTG AAA GCC GGC GAC GCG
     Glu Thr Glu Ile Arg Ala Ala Gln Ala Gly Thr Val Arg Gly Ile Ala Val Lys Ala Gly Asp Ala
                            Biotag™ forward priming site
                           |                              |
483  GTG GCG GTC GGC GAC ACC CTG ATG ACC CTG GCG GGC TCT GGA TCC GAT CTG TAC GAC GAT GAC GAT
     Val Ala Val Gly Asp Thr Leu Met Thr Leu Ala Gly Ser Gly Ser Asp Leu Tyr Asp Asp Asp Asp
                                                                              EK recognition site
549  AAG GGA ATT GAT CCC TTG AGG       AAGGGCGAGCT CAGATCCGGC TGCTAACAAA GCCCGAAAGG
     Lys Gly Ile Asp Pro  GGG GAG ...
     |                   |
      EK cleavage site       T7 reverse priming site
611  AAGCTGAGTT GGCTGCTGCC ACCGCTGAGC AATAACTAGC
```

Fig. 19

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pcDNA6/Biotag-DEST™ vector by recombination. Non-shaded regions are derived from the pcDNA6/Biotag-DEST™ vector.
- Bases 1191 and 2853 of the pcDNA6/Biotag-DEST™ sequence are marked.
- The biotin binding site is labeled with a *.
- Potential stop codons are underlined.

```
                      CAAT                                           TATA          3'end of CMV promoter        Putative transcriptional start
761   CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA

                                        T7 promoter/priming site
841   CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC GTTTAAACTT AAGCTTACC ATG
                                                Biotag™                                                           Met

      Gly Ala Gly Thr Pro Val Thr Ala Pro Leu Ala Gly Thr Ile Trp Lys Val Leu Ala Ser Glu Gly
923   GGC GCC GGC ACC CCG GTG ACC GCC CCG CTG GCG GGC ACT ATC TGG AAG GTG CTG GCC AGC GAA GGC

      Gln Thr Val Ala Ala Gly Glu Val Leu Leu Ile Leu Glu Ala Met Lys Met Glu Thr Glu Ile Arg
989   CAG ACG GTG GCC GCA GGC GAG GTG CTG CTG ATT CTG GAA GCC ATG AAG ATG GAA ACC GAA ATC CGC
                                                                        Biotin binding site

      Ala Ala Gln Ala Gly Thr Val Arg Gly Ile Ala Val Lys Ala Gly Asp Ala Val Ala Val Gly Asp
1055  GCC GCG CAA GCC GGG ACC GTG CGC GGT ATC GCG GTG AAA GCC GGC GAC GCG GTG GCG GTC GGC GAC
      Biotag™ forward priming site                                    EK recognition site           EK cleavage site

      Thr Leu Met Thr Leu Ala Gly Ser Gly Ser Asp Leu Tyr Asp Asp Asp Asp Lys Val His Gln Thr
1121  ACC CTG ATG ACC CTG GCG GGC TCT GGA TCC GAT CTG TAC GAC GAT GAT GAT AAG GTA CAT CAA ACA
                                                                                   CAT GTA GTT TGT

      1191                                                             2853
      Ser Leu Tyr Lys Lys Ala Gly ... ... ***
1187  AGT T▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓GENE▓▓▓▓▓▓▓▓▓▓CTTGTACA AAGTGGTGAT AATTAATTAA
      TCA AAC ATG TTT ▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓T TTCACCACTA TTAATTAATT
             attB1                                              attB2
                                        BGH reverse priming site

2881  GATCTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT
```

Fig. 20

Flow Chart

The flow chart below outlines the experimental steps necessary to clone and express your blunt-end PCR product.

```
            ┌─────────────────────────────────────┐
            │      Determine strategy for PCR      │
            └─────────────────────────────────────┘
                              │
                              ▼
      ┌─────────────────────────────────────────────┐
      │         Produce blunt-end PCR product        │
      │      using properly designed PCR primers     │
      └─────────────────────────────────────────────┘
                              │
                              ▼
   ┌──────────────────────────────────────────────────┐
   │              TOPO® Cloning Reaction:              │
   │  Mix together PCR product and pcDNA6/Biotag™/D-TOPO® │
   └──────────────────────────────────────────────────┘
                              │  Incubate 5 minutes
                              ▼  at room temperature
          ┌─────────────────────────────────────┐
          │    Transform into TOP10 E. coli cells │
          └─────────────────────────────────────┘
                              │
                              ▼
          ┌─────────────────────────────────────┐
          │       Select and analyze colonies    │
          └─────────────────────────────────────┘
                              │
                              ▼
        ┌───────────────────────────────────────┐
        │  Prepare purified plasmid for transfection │
        └───────────────────────────────────────┘
                              │
                              ▼
      ┌─────────────────────────────────────────────┐
      │        Transfect mammalian cell line and      │
      │      test for expression of gene of interest  │
      └─────────────────────────────────────────────┘
```

Fig. 21

```
                    CAAT                                        TATA      5'end of CMV promoter
                   ┌──────┐                                   ┌────┐                    ┌─────────────────────────┐
761  CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
                                                                              ▼    Putative transcriptional start
                                                                                           ─────────────►

                               T7 promoter/priming site
                              ┌─────────────────────────┐
841  CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC GTTTAAACTT AAGCTTACC ATG
                                                                                                Met
                                        Biotag™
923  GGC GCC GGC ACC CCG GTG ACC GCC CCG CTG GCG GGC ACT ATC TGG AAG GTG CTG GCC AGC GAA GGC
     Gly Ala Gly Thr Pro Val Thr Ala Pro Leu Ala Gly Thr Ile Trp Lys Val Leu Ala Ser Glu Gly

989  CAG ACG GTG GCC GCA GGC GAG GTG CTG CTG ATT CTG GAA GCC ATG AAG ATG GAA ACC GAA ATC CGC
     Gln Thr Val Ala Ala Gly Glu Val Leu Leu Ile Leu Glu Ala Met Lys Met Glu Thr Glu Ile Arg
                                                                            *
                                                                            Biotin binding site
     ─────────────────────────────────────────────────────────────────────────────────────────
1055 GCC GCG CAG GCC GGG ACC GTG CGC GGT ATC GCG GTG AAA GCC GGC GAC GCG GTG GCG GTC GGC GAC
     Ala Ala Gln Ala Gly Thr Val Arg Gly Ile Ala Val Lys Ala Gly Asp Ala Val Ala Val Gly Asp
     Biotag™ forward priming site                                              Asp7181  Kpn I
     ┌──────────────────────────┐                                             ┌──────────┐
1121 ACC CTG ATG ACC CTG GCG GGC TCT GGA TCC GAT CTG TAC GAC GAT AAG GTA CCT AGG ATC
     Thr Leu Met Thr Leu Ala Gly Ser Gly Ser Asp Leu Tyr Asp Asp Asp Lys Val Pro Arg Ile
                                                      EK recognition site        EK cleavage site
                                                      Xba I           Acc I
1187 CAG TGT GGT GGA ATT GAT CCC TTT ACC         AAGGGCG TCGAGTCTAG AGGGCCCGTT TAAACCCGCT
     Gln Cys Gly Gly Ile Asp Pro Phe Thr
                BGH reverse priming site
              ┌─────────────────────────┐
1251 GATCAGCCTC GACTGTGCCT TCTAGTTGCC AGCCATCTGT TGTTTGCCCC
```


Fig. 22

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pMT/Biotag™-DEST vector by recombination. Non-shaded regions are derived from the pMT/Biotag™-DEST vector.
- Bases 1135 and 2797 of the pMT/Biotag™-DEST sequence are marked.
- The biotin binding site is labeled with a *.
- Potential stop codons are underlined.
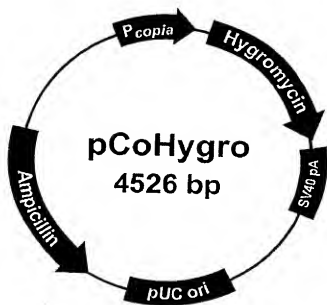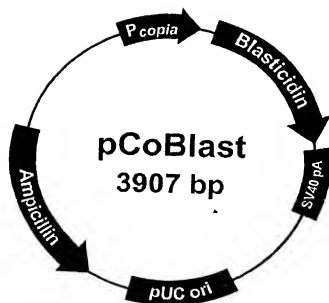
```
       5' end of metallothionein promoter                                    Metal regulatory region
411  CGTTGCAGGA CAGGATGTGG TGCCCGATGT GACTAGCTCT TTGCTGCAGG CCGTCCTATC CTCTGGTTCC GATAAGAGAC CCAGAACTCC

                                                                                        Metal regulatory
                                                                                        region
501  GGCCCCCCAC CGCCCACCGC CACCCCCATA CATATGTGGT ACGCAAGTAA GAGTGCCTGC GCATGCCCCA TGTGCCCCAC CAAGACTTTT

     region                                                    Metal regulatory regions
591  GCATCCCATA CAAGTCCCCA AAGTGGAGAA CCCAACCAAT TCTTCGCGGG CAGAACAAAA GCTTCTGCAC ACGTCTCCAC TCGAATTTGG

                                                                                           TATA
681  AGCCGGCCGG CGTGTGCAAA AGAGGTGAAT CGAACGAAAG ACCCGTGTGT AAAGCCGCGT TTCCAAAATG TATAAAACCG AGAGCATCTG
                       Metal regulatory region             Metal regulatory regions

            Start of transcription
771  GCCAATGTGC ATCAGTTGTG GTCAGCAGCA AAATCAAGTG AATCATCTCA GTGCAACTAA AGGGGGGATC TAGCGTTTAA ACTTAAGCTT
                                                              Biotag™

          Met Gly Ala Gly Thr Pro Val Thr Ala Pro Leu Ala Gly Thr Ile Trp Lys Val Leu Ala Ser Glu
861  ACC ATG GGC GCC GGC ACC CCG GTG ACC GCC CCG CTG GCG GGC ACT ATC TGG AAG GTG CTG GCC AGC GAA

     Gly Gln Thr Val Ala Ala Gly Glu Val Leu Leu Ile Leu Glu Ala Met Lys Met Glu Thr Glu Ile Arg
930  GGC CAG ACG GTG GCC GCA GGC GAG GTG CTG CTG ATT CTG GAA GCC ATG AAG ATG GAA ACC GAA ATC CGC
                                                                             *
                                                                             Biotin binding site

     Ala Ala Gln Ala Gly Thr Val Arg Gly Ile Ala Val Lys Ala Gly Asp Ala Val Ala Val Gly Asp Thr
999  GCC GCG CAG GCC GGG ACC GTG CGC GGT ATC GCG GTG AAA GCC GGC GAC GCG GTG GCG GTC GGC GAC ACC

                                                    EK recognition site    EK cleavage site         1135
     Leu Met Thr Leu Ala Gly Ser Gly Ser Asp Leu Tyr Asp Asp Asp Asp Lys Val His Gln Thr Ser Leu
1068 CTG ATG ACC CTG GCG GGC TCT GGA TCC GAT CTG TAC GAC GAT GAC GAT AAG GTA CAT CAA ACA AGT TTG
                                                                             CAT GTA GTT TGT TCA AAC
                                                                       2797
     Tyr Lys Lys Ala Gly ... ... ***                             |
1137 AAG TAA AAA GCA GCT ...  GENE    ...  AGCTTCCTTG TACAAAGTGG TGATAATTAA TTAAGATCTA GAGGGCCCGT
     ATG TTT ... ... ... ... ...  ... ... ... ... ...  ...  ...  TTCACC ACTATTAATT
     attB1                                        attB2
```

continued on next page

Fig. 23

pCoHygro
4526 bp

P copia
Hygromycin
SV40 pA
pUC ori
Ampicillin

Fig. 24

Fig. 25